Spot Test Method Convenient for Detecting the Lipid Content in Column Chromatography

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Spot Test Method Convenient for Detecting the Lipid Content in Column Chromatography

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A spot test method is described as a simple and quick method of estimating lipid content in fractions eluted by column chromatography. Standard spots were prepared by spotting drops of chloroform solutions of total lipids in known concentrations on a thin layer chromatographic (TLC) plate. In the course of column separation experiment, one drop of the eluate at any time was spotted on the TLC plate, and the lipid content of the spot was readily estimated by comparison with the standard spots. The method is useful for instantaneous estimation of the lipid concentration and for control of the volume and the composition of eluents in the column chromatography.

I. INTRODUCTION

In separation experiments of lipid components by means of column chromatography, a quantitative analysis of the lipid concentration for each fraction eluted is usually carried out together after the elution experiment. If it is possible, however, with a simple method to check the lipid concentration in the eluate at any moment during the elution, the composition and the volume of the eluents can be controlled efficiently in the course of the elution.

Lipid concentrations of spots on thin-layer chromatographic (TLC) plates are estimated usually by measurement of the spot area and density or by gravimetric, spectrophotometric, colorimetric and radiometric techniques.[1] In practice these techniques are not necessarily convenient for the frequent use at any time during the elution. Some authors adopted a comparison method of the spot density in their experiments. For a glycolipid analysis, Hakomori[2] adopted a comparison method of spot densities on serial dilutions of the unknown samples with those of standard samples, the detailed explanation being lacking.

In the present study a simple and quick method is proposed to estimate the lipid concentration by comparison of the color intensity of the eluate spotted on TLC plates with those of standard spots whose lipid concentrations are known. The method is useful for the instantaneous estimation of the lipid concentration of the eluate for controlling volume and composition of the eluent in column chromatography.

II. EXPERIMENTAL

1. Procedure of Comparison of Spot Intensity

In order to prepare standard spots, chloroform solutions were made up of total lipids

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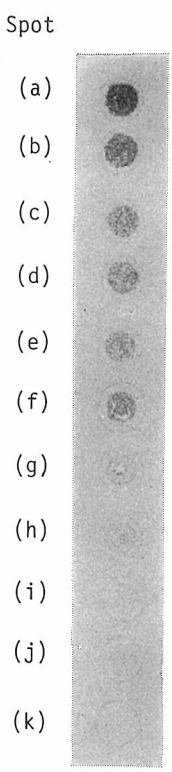

Fig. 1 Reference Spots made from total lipid solutions in various concentrations.
The total lipid is extracted from rat kidney.
Thin-layer plate: Kieselgel G nach Stahl.
Indicator: phosphomolybdic acid.
The total lipid extracted from rat kidney is dissolved in chloroform in various concentrations indicated beside the photograph, 0.02 mls of the lipid solutions being spotted on a thin-layer chromatographic plate.

including neutral lipids and phospholipids in various concentrations: 1, 0.5, 0.4, 0.3, 0.25, 0.2, 0.15, 0.1, 0.05, 0.0375, and 0.025 mg of lipids per millilitre of chloroform.

These individual solutions (0.02 ml, corresponding approximately to one drop) were spotted on a TLC plate made of Kieselgel G nach Stahl. The spots were developed with an indicators positive for all lipids such as phosphomolibdic acid, sulfuric acid and iodine vapor, the results being shown in Fig. 1, henceforth referred to as Reference Spots.

In the course of column elution, one drop of the eluate falling down from the bottom tip of the column, usually about 0.02 ml in volume, was spotted on the TLC plate occasionally. After the color development, the intensity of the spot was compared by the eye with Reference Spots shown in Fig. 1 to estimate the lipid concentration. These Reference Spots prepared from the total lipid solution were available in practice for all lipids, though the color tone and the intensity of individual spots of the eluate were varied slightly from lipid to lipid.

2. Other Possibilities of Spotted Plates and Indicators

The use of TLC plates was most satisfactory for the estimation of the lipid concentra-

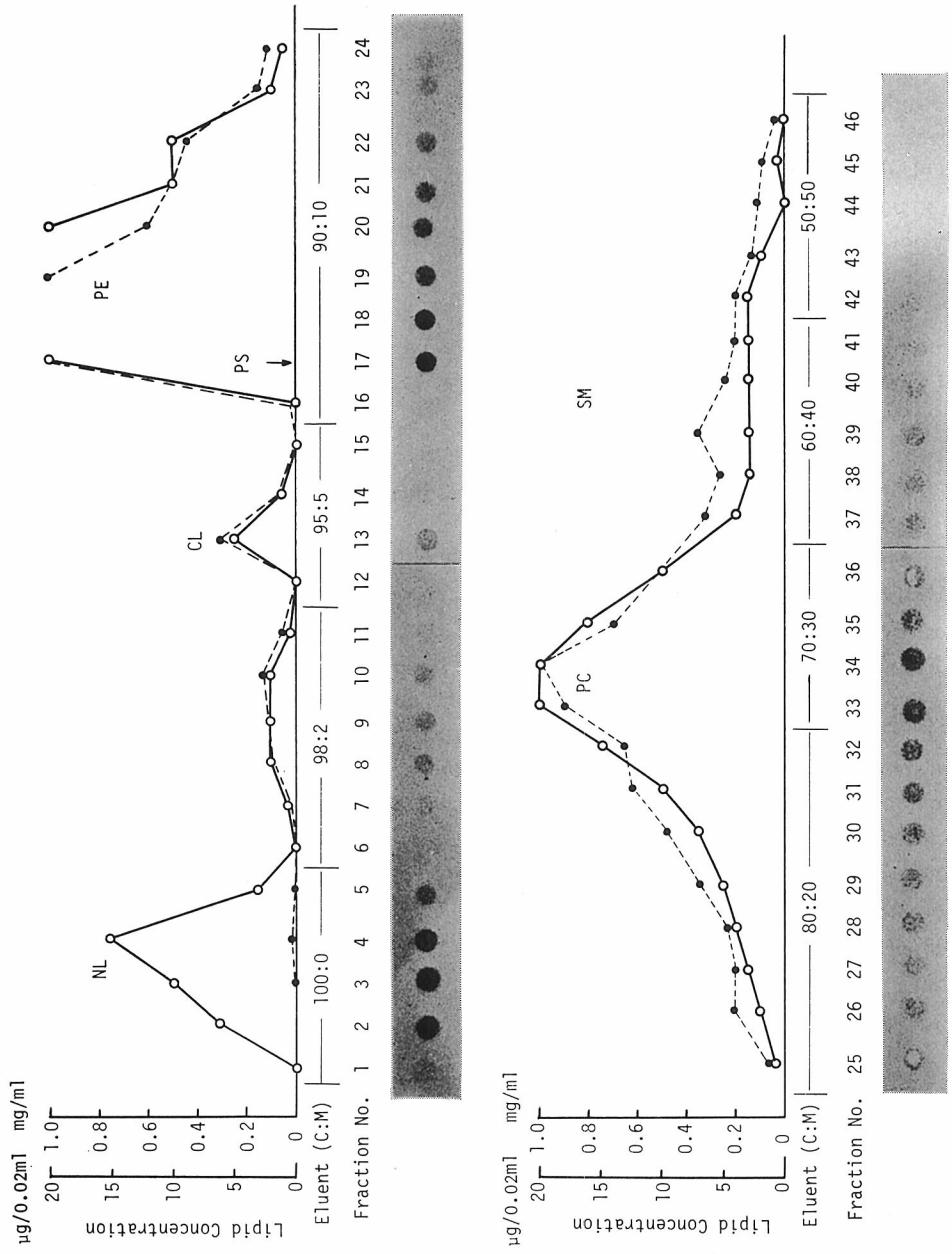

Fig. 2 Comparison of lipid concentration estimated by the two methods for column eluates.

Total lipids of rat kidney are separated into fractions corresponding to eluents in varied composition of chloroform-methanol (C-M) mixtures by means of a silicic acid column. Lipid concentrations of each fraction are estimated by the spot test method (—○—) and by the quantitative analysis of phosphorus content (- - -●- - -). Main components: NL, neutral lipids; CL, cardiolipin; PS, phosphatidyl serine; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin.

tion, because the TLC plates were found to be sensitive to the intensities of the spots developed by various indicators as expected. By the use of some filter papers, simpler methods were also available with some limitations shown below.

The filter papers in conventional use were also usable for this spot test. After spraying the indicators over the spots on the filter paper, the colors of the indicators were readily developed by simply heating with an electric heater. Iodine vapor was always positive to all classes of lipid on the filter papers, whereas phosphomolybdic acid was less sensitive or, in some instances, insensitive to phosphatidyl inositol, phosphatidyl choline and sphingomyelin. Hence the reproducibility of the spot intensity on the filter paper was too poor to be used for quantitative estimation of lipid concentration.

Glass fibre filter papers, Teflon fibre filter papers and DEAE cellulose fibre filter papers were found to be usable, but less sensitive to the lipid concentration as compared with the TLC plates. It is thus most advisable for the spot test to use TLC plates, though the filter papers are more convenient to use.

III. APPLICATIONS

An application of the present method is given in Fig. 2, which shows the comparison of lipid concentrations estimated by the spot test method with those by a quantitative analysis of phosphorus content proposed by Bartlett[3] for each fraction eluted by a silicic acid column for the resolution of rat kidney lipids. As can be seen in Fig. 2, the values by the two methods are in substantial agreement with each other. Since the main components of Fractions Nos. 2 to 5 in Fig. 2 are non-phosphorous neutral lipids, it is reasonable that the spot test method should give considerably high values of lipid concentration, whereas the phosphorous analysis showed very low values of the contents.

For the non-phosphorous lipids, Bartlett's method is of no use to estimate lipid concentration. In such a case, the spot test method should be compared with the dry-weight method. Figure 3 illustrates such comparisons for the neutral lipid fractions. Also in this case, the agreement is quantitatively satisfactory.

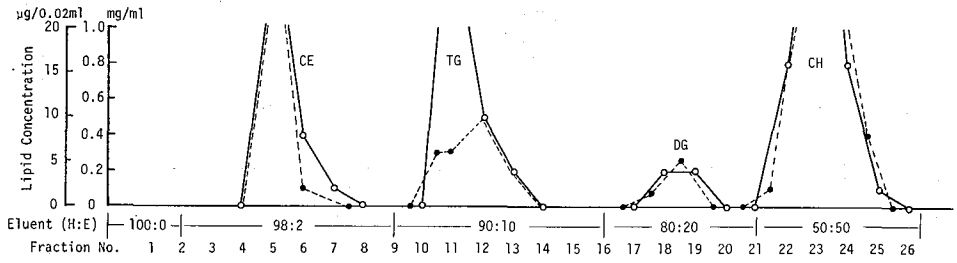

Fig. 3 Comparison of neutral lipid concentration estimated by the two methods for column eluates.

Neutral lipid fractions are further separated into fractions corresponding to eluents in varied composition of hexane-diethyl ether (H-E) mixtures by means of a silica gel column. The neutral lipid concentrations of each fraction are estimated by the spot test method (—○—) and by a dry-weight method (- - -●- - -). Main components: CE, cholesterol esters; TC, triglycerides; DG, diglycerides; CH, cholesterol.

From these examples it was found that the spot test method is applicable to semi-quantitative analysis as well as qualitative inspection of lipid concentrations. In practice of column elution, the eluent should be changed stepwise to the subsequent stage with respect to the composition when the lipid concentration of the eluate is decreased down to about 1 μg per drop of about 0.02 ml. The composition and the volume of the eluents thus can be changed efficiently in the course of the elution experiment.

Accuracy of the spot test method is associated with the contrast of color intensity among the spots. In practice, the accuracy of this method is too low to be used at higher concentrations of more than 0.5 mg/ml corresponding to Spots (a) and (b) in Fig. 1. In Figs. 2 and 3, the data are cut off for Fractions in such higher concentrations. In the optimal range of 0.1 to 0.3 mg/ml corresponding to Spots (d) to (h) in Fig. 1, the lipid concentration can readily be estimated within errors of about 20%.

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